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PATENT COOPERATION TREATY

PCT

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Article 36 and Rule 70)

Applicant's or agent's file reference 0000053691	**FOR FURTHER ACTION**	See Notification of Transmittal of International Preliminary Examination Report (Form PCT/IPEA/416)
International application No. PCT/EP 03/07028	International filing date *(day/month/year)* 02.07.2003	Priority date *(day/month/year)* 05.07.2002
International Patent Classification (IPC) or both national classification and IPC C12N15/80		
Applicant BASF AKTIENGESELLSCHAFT et al.		

1. This international preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 36.

2. This REPORT consists of a total of 10 sheets, including this cover sheet.

   ☒ This report is also accompanied by ANNEXES, i.e. sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications made before this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions under the PCT).

   These annexes consist of a total of 7 sheets.

3. This report contains indications relating to the following items:

   - I ☒ Basis of the opinion
   - II ☐ Priority
   - III ☒ Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
   - IV ☒ Lack of unity of invention
   - V ☒ Reasoned statement under Rule 66.2(a)(ii) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
   - VI ☐ Certain documents cited
   - VII ☐ Certain defects in the international application
   - VIII ☐ Certain observations on the international application

Date of submission of the demand	Date of completion of this report
29.01.2004	19.11.2004
Name and mailing address of the international preliminary examining authority: European Patent Office D-80298 Munich Tel. +49 89 2399 - 0 Tx: 523656 epmu d Fax: +49 89 2399 - 4465	Authorized Officer Steffen, P Telephone No. +49 89 2399-7307

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No. PCT/EP 03/07028

I. Basis of the report

1. With regard to the **elements** of the international application *(Replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to this report since they do not contain amendments (Rules 70.16 and 70.17))*:

Description, Pages

1-97 as originally filed

Claims, Numbers

1-20 received on 02.11.2004 with letter of 02.11.2004

Drawings, Sheets

1/1 as originally filed

2. With regard to the **language**, all the elements marked above were available or furnished to this Authority in the language in which the international application was filed, unless otherwise indicated under this item.

These elements were available or furnished to this Authority in the following language: , which is:

☐ the language of a translation furnished for the purposes of the international search (under Rule 23.1(b)).

☐ the language of publication of the international application (under Rule 48.3(b)).

☐ the language of a translation furnished for the purposes of international preliminary examination (under Rule 55.2 and/or 55.3).

3. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international preliminary examination was carried out on the basis of the sequence listing:

☒ contained in the international application in written form.

☒ filed together with the international application in computer readable form.

☐ furnished subsequently to this Authority in written form.

☐ furnished subsequently to this Authority in computer readable form.

☐ The statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.

☐ The statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished.

4. The amendments have resulted in the cancellation of:

☐ the description, pages:

☐ the claims, Nos.:

☐ the drawings, sheets:

5. ☐ This report has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed (Rule 70.2(c)).

   (Any replacement sheet containing such amendments must be referred to under item 1 and annexed to this report.)

6. Additional observations, if necessary:

III. Non-establishment of opinion with regard to novelty, inventive step and industrial applicability

1. The questions whether the claimed invention appears to be novel, to involve an inventive step (to be non-obvious), or to be industrially applicable have not been examined in respect of:

   ☐ the entire international application,

   ☒ claims Nos. 9-20

   because:

   ☒ the said international application, or the said claims Nos. 9-20 relate to the following subject matter which does not require an international preliminary examination (specify):

   see separate sheet

   ☐ the description, claims or drawings *(indicate particular elements below)* or said claims Nos. are so unclear that no meaningful opinion could be formed *(specify)*:

   ☐ the claims, or said claims Nos. are so inadequately supported by the description that no meaningful opinion could be formed.

   ☐ no international search report has been established for the said claims Nos.

2. A meaningful international preliminary examination cannot be carried out due to the failure of the nucleotide and/or amino acid sequence listing to comply with the standard provided for in Annex C of the Administrative Instructions:

   ☐ the written form has not been furnished or does not comply with the Standard.

   ☐ the computer readable form has not been furnished or does not comply with the Standard.

IV. Lack of unity of invention

1. In response to the invitation to restrict or pay additional fees, the applicant has:

   ☐ restricted the claims.

   ☐ paid additional fees.

   ☐ paid additional fees under protest.

   ☒ neither restricted nor paid additional fees.

2. ☐ This Authority found that the requirement of unity of invention is not complied with and chose, according to Rule 68.1, not to invite the applicant to restrict or pay additional fees.

3. This Authority considers that the requirement of unity of invention in accordance with Rules 13.1, 13.2 and 13.3 is

☐ complied with.

☒ not complied with for the following reasons:

see separate sheet

4. Consequently, the following parts of the international application were the subject of international preliminary examination in establishing this report:

☐ all parts.

☒ the parts relating to claims Nos. 1-8 .

V. Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Yes:	Claims	6
	No:	Claims	1-5,7,8
Inventive step (IS)	Yes:	Claims	
	No:	Claims	1-8
Industrial applicability (IA)	Yes:	Claims	1-8
	No:	Claims	

2. Citations and explanations

see separate sheet

Re Item III

Non-establishment of opinion with regard to novelty, inventive step and industrial applicability

In a communication, dated from 05.07.2004, the IPEA indicated with the below reasoning of Item IV the presence of multiple inventions for the present application (five in all) and invited the applicants either to restrict or to pay additional fees, in accordance with Rule 68.2 PCT. None of the additional fees have been duly paid in the prescribed time limits according to the provisos of Rules 68.2 and 68.3, (a) and (b) PCT. Accordingly, the present opinion is established under Article 34(3)(c) and Rule 68.5 PCT on invention 1 (as set out below in Item IV) which is considered to be the main invention, being that which is first mentioned in the claims. Also since no further fees had been paid in the time limit due, let alone under protest, no review of the non-unity finding is to be made at the current stage of the international examination. Therefore the present opinion does only relate to the below mentioned invention 1 e.g. to claims 1-8 but **does not relate** the other inventions 2-5 as mentioned below in Item IV (e.g new claims 9-20 and former claims 9-22).

Re Item IV

Lack of unity of invention

The IPEA found the following five inventions for the present application:

Invention 1. Claims: 1-8

Plasmid vectors for targeted integration of filamentous fungi and various embodiments thereof.

Invention 2: claim 9 (completely) and claims 10-22 (all partly)

A selection marker comprising a nucleic acid encoding a polyketide synthase or fragments and functional equivalents wherein the pks gene is relating to SEQ ID NO's: 1-8 e.g. the pks of Fusarium graminearum as isolated in the present application. Furthermore plasmid vectors for targeted transformation of filamentous fungi and expression cassettes comprising said DNA and use of the said nucleic acid as marker for targeted transformation of filamentous fungi as well as in colour-selectable transformation methods for filamentous fungi.

Invention 3: claims 10-22 (all partly)

Plasmid vectors for targeted transformation of filamentous fungi and expression cassettes comprising a selection marker comprising a nucleic acid encoding a pks fragment as set out in SEQ ID NO's: 9 and 10 and use of the said nucleic acid as marker for targeted transformation of filamentous fungi as well as in colour-selectable transformation methods for filamentous fungi.

Invention 4: claims 10-22 (all partly)

Plasmid vectors for targeted transformation of filamentous fungi and expression cassettes comprising a selection marker comprising a nucleic acid encoding a pks fragment as set out in SEQ ID NO's: 11 and 12 and use of the said nucleic acid as marker for targeted transformation of filamentous fungi as well as in colour-selectable transformation methods for filamentous fungi.

Invention 5: claims 10-22 (all partly)

Plasmid vectors for targeted transformation of filamentous fungi and expression cassettes comprising a selection marker comprising a nucleic acid encoding a pks fragment as set out in SEQ ID NO: 13 and use of the said nucleic acid as marker for targeted transformation of filamentous fungi as well as in colour-selectable transformation methods for filamentous fungi.

The present application relates to plasmid vectors for targeted transformation of filamentous fungi (claims 1-8) and to selection markers comprising a nucleic acid sequence encoding a polyketide synthetase (pks) fragment as well as further embodiments thereof (claims 9-22) where the plasmid vectors of claim 1-8 do not comprise the said selection marker. Concerning the pks selection marker, this can be a polyketide synthase (pks) isolated from Fusarium graminearum (SEQ ID's 1-8) or of other origin (SEQ ID NO's 9-13). They are implicated in pigment biosynthesis and used in targeted transformation of filamentous fungi. This allows for (absence of) colour selection on the plates when there is an genomic integration with disruption of the targeted polyketide synthase gene. Various use of the pks as well as use of pks from other organisms are equally claimed.

Plasmid vectors for targeted transformation of filamentous fungi are known in the art.

For example Feng et al. (a) (2001, Infection and Immunity, vol. 69(3), pp. 1781-1794)

disclose an integration vector pBF9 for the (filamentous) fungus Wangiella dermatitidis.

Tsai et al. (1998, Journal of Bacteriology, vol. 180(12), pp. 3031-3038) disclose the cloning of the alb1 polyketide synthase gene of Aspergillus fumigatus (filamentous). Also disclosed are knock-out constructs and selection on a colour-based assay since transformed hosts have no coloured but white conidia. Complementation is also done in order to restore colour on albino phenotypes. alb1 has 46% ID with SEQ 6, 84.2% with SEQ 8 and comprises a sequence of 100 aa around SEQ 8 homolog of 88% aa ID. The vector used for transformation is a pBC-KS with a 2.8 kbp hph cassette from pAN7-1 (Punt et al., 1987, Gene, vol. 56, pp. 117-124) and thus fits with the requirements of claim 1.

In view of the above prior art citations disclosing plasmid vectors for targeted transformation/integration for filamentous fungi, in view of the fact that the plasmid vectors as claimed in claims 1-8 do not employ the selection markers as claimed in claims 9-19 and that these markers are not needed for the said plasmids to fulfill their function and considering also the description on page 3, lines 9-13 and page 10, lines 8-11, there can be seen no special technical feature between the subject-matter claimed, e.g. plasmid vector and pks synthase as selection marker, making a link to a common inventive concept according to Article 34(3)(c) PCT and Rules 13.1 and 13.2 PCT.

Moreover is the use of polyketide synthase or fragments thereof as colour-based selection marker for targeted transformation of filamentous fungi is well established in the art and has been described inter alia in Feng et al. (a) (supra), Tsai et al. (supra), Feng et al. (b) (1995, Journal of Bacteriology, vol. 180(12), pp. 6246-6254), Ye et al. (1999, Current Genetics, vol. 36(4), pp. 241-247), Mayorga et al. (1990, Genetics, 1990, vol. 126(1), pp. 73-79 and Chang et al. (1995, MGG, vol. 248(3), pp.270-277). These documents describe knock-out experiments with various pks genes and selecting the genomic integrants by colour selection. Feng et al. (a) (supra), Tsai et al. (supra) and Mayorga et al. (supra) describe also the restoration of the colour phenotype when reintroducing the pks gene in the knock-out background. Moreover does the pks of Tsai et al. (supra) encode a protein of 50% identity with the SEQ ID 6, and 84% identity with the SEQ ID 8, which is the aa 523-599 fragment of SEQ ID 6. The other pks genes as appearing in the use claims are known in the art e.g. from Feng et al. (a) (supra), Ye et al. (supra) (SEQ's 9/10), Feng et al. (b) (supra) (SEQ's 11/12) and Mayorga et al. (supra) (SEQ 13). Also these genes and the encoded proteins have an identity with SEQ ID 6 of 39-42% which is in no way higher than those between SEQ ID 6 and the pks of Tsai et al. (supra). Due also to the identities many of the pks-related method claims are anticipated in the art, so for example Tsai et al. (supra) and Mayorga et al. (supra) which clearly anticipate the method claims.

In view of the above considerations it is considered, that the pks-related inventions (claims 9-22) do not fulfill the requirements of unity of invention as set out in Article 34(3)(c) PCT and Rules 13.1 and 13.2 PCT, because there can be found no special technical feature linking the different groups of inventions 2-5 to a single and common inventive concept. Hence the IPEA considers that there is the presence of five different inventions as mentioned above.

Re Item V

Reasoned statement under Rule 66.2(a)(ii) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

The following documents D1-D6 are referred to in this communication; the numbering will be adhered to in the rest of the procedure:

D1: FENG B ET AL: 'Molecular cloning and characterization of WdPKS1, a gene involved in dihydroxynaphthalene melanin biosynthesis and virulence in Wangiella (Exophiala) dermatitidis.' INFECTION AND IMMUNITY. UNITED STATES MAR 2001, vol. 69, no. 3, March 2001 (2001-03), pages 1781-1794, XP002247661 ISSN: 0019-9567

D2: SHIBAYAMA MAYUMI ET AL: 'Suppression of tandem-multimer formation during genetic transformation of the mycotoxin-producing fungus Penicillium paxilli by disrupting an orthologue of Aspergillus nidulans uvsC.' CURRENT GENETICS. UNITED STATES OCT 2002, vol. 42, no. 1, October 2002 (2002-10), pages 59-65, XP002247662 ISSN: 0172-8083

D3: PUNT P J ET AL: 'TRANSFORMATION OF ASPERGILLUS BASED ON THE HYGROMYCIN B RESISTANCE MARKER FROM ESCHERICHIA COLI' GENE, ELSEVIER BIOMEDICAL PRESS. AMSTERDAM, NL, vol. 56, 1987, pages 117-124, XP001093695 ISSN: 0378-1119

D4: TSAI H F ET AL: 'The developmentally regulated alb1 gene of Aspergillus fumigatus: its role in modulation of conidial morphology and virulence.' JOURNAL OF BACTERIOLOGY. UNITED STATES JUN 1998, vol. 180, no. 12, June 1998 (1998-06), pages 3031-3038, XP002264291 ISSN: 0021-9193

D5: ZHANG A ET AL: 'Efficient disruption of a polyketide synthase gene (pks1) required for melanin synthesis through Agrobacterium-mediated transformation of Glarea lozoyensis.' MOLECULAR GENETICS AND GENOMICS: MGG. GERMANY FEB 2003, vol. 268, no. 5, February 2003 (2003-02), pages 645-655, XP002264294 ISSN: 1617-4615

D6: LINNEMANNSTÖNS PIA ET AL: 'The polyketide synthase gene pks4 from Gibberella fujikuroi encodes a key enzyme in the biosynthesis of the red pigment bikaverin.' FUNGAL GENETICS AND BIOLOGY: FG & B. UNITED STATES NOV 2002, vol. 37, no. 2, November 2002 (2002-11), pages 134-148, XP002264295 ISSN: 1087-1845

Invention 1 (claims 1-8) of the present application relates to transformation/integration vectors for filamentous fungi carrying an ORI, an antibiotic selection marker and a hygromycin resistance cassette, these three elements having less than 4500 bp in size, and fungal sequences for homologous recombination.

D1 discloses an integration vector pBF9 for the (filamentous) fungus Wangiella dermatitidis (see also description, page 13, lines 34-38). It is 6 kbp in size with an integration fragment of polyketide synthase of 0.8 kbp, the ORI is from pBKS, there is a Cm resistance and hph (hygromycin) resistance cassette which comprises a Ptrc promoter but no terminator element (reference 6 of D1) and from the vector illustration these three elements do not make more than 4.2 kbp (pBKS ORI is 0.67 kbp according to manufacturer data). Due to a lack of a terminator this plasmid does not anticipate novelty of claims 1-3.

D4 discloses an integration vector pRGD12 for the filamentous Aspergillus fumigatus in the alb1 polyketide synthase gene. The vector used for transformation is a pBC-KS with a 2.8 kbp hph cassette from pAN7-1 which has a gdp promoter and trpC terminator (D3). While it is appreciated, that the whole hph cassette of pAN7-1 is 3.2 kb in length (Figure 1 of D3), there is no doubt that in the vector of D4 only a 2.8 kb fragment of this cassette is integrated. This is clear from the text (page 3032, right column, lines 11-13) which stipulates that a "*1.5-kb MluI-AvrII fragment was replaced with a **2.8 kb hph cassette** from pAN7-1*" and also from Figure 4A, where the hph cassette is between the destroyed MluI/**HindIII** and **SmaI**/AvrII restriction sites (e.g. not between the full-length hph cassette restriction sites **HindIII** and **EcoRI**) and in which the scale clearly indicates that the black box corresponding to the **hph cassette** (HindIII-SmaI) is of the length **of 2.8 kb** and not of the length of 3.2 kb. Furthermore is pBC-KS a pUC derived vector having a **Cm-resistance gene of 0.67 kb** and a **pUC ori of 0.67 kb**. All in all these three elements make 4.14 kb e.g. below the 4.5 kb as claimed in claim 1. In addition, the vector of D4 comprises the alb1 fragment for integration between the lac promoter and terminator of pBC-KS. Hence D4 anticipates novelty of claims 1-5 and 7, 8.

In conclusion claims 1-5, 7 and 8 lack novelty contrary to Article 33(2) PCT and are

therefore also not based on inventive step, contrary to the requirements of Article 33(3) PCT.

Claim 6 relates to a vector of claims 1-5 with a specific combination of well-known promoter and terminator elements flanking the hygromycin resistance gene a combination not disclosed in D1, D3 and D4. Hence claim 6 is novel according to Article 33(2) PCT.

However, all vectors as set out in D1, D3 and D4 have a promoter and terminator flanking the hph cassette for expression and transcription termination. There are however no technical effects linked to such a specific combination of promoter and terminator as set out in claim 6 e.g. it does not appear that such combination solves a true technical problem but it rather appears that it combines use of two different well-known elements (description, pages 6-7) in an fortuitous manner. Such fortuitous shuffling of known elements with their known effects is, unless there is creation of a technical effect that solves a technical problem, obvious to any skilled person and cannot contribute to reinstate inventive step vis-à-vis D4.

Therefore claim 6 lacks inventive step, contrary to Article 33(3) PCT.

Re Item VI
Certain documents cited

D2, D5 and D6, cited as P documents in the ISR are relevant for novelty and/or inventive step of claims 1-8, should the claimed priority be found not to be valid.